WEST Search History

DATE: Saturday, August 24, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB; PLUR=YES; OP=ADJ		
L13	L12 and @ad<20000621	412	L13
L12	L11 and l7	593	L12
L11	L10 and l9 and l8	1074	L11
L10	nucleic acid or polynucleotide or nucleotide or vector	151538	L10
L9	human or man	368805	L9
L8	Cytochrome p450 or cytochrome p 450 or cytochrome p450 NADPH reductase or cytochrome p450 reductase	1840	L8
L7	L6 or l5 or l4 or l3 or l2 or l1	24683	L7
L6	(((536/23.2)!.CCLS.))	4391	L6
L5	(((435/320.1)!.CCLS.))	12435	L5
L4	(((435/252.3)!.CCLS.))	5854	L4
L3	(((435/189)!.CCLS.))	848	L3
L2	(((435/183)!.CCLS.))	1639	L2
L1	((435/6)!.CCLS.)	11280	L1

END OF SEARCH HISTORY

WEST

Generate Collection | Print

Search Results - Record(s) 1 through 10 of 412 returned.

☐ 1. Document ID: US 20020102602 A1

L13: Entry 1 of 412 File: PGPB Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020102602
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020102602 A1

TITLE: COMPOSITIONS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER AND METHODS FOR THEIR USE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020099197 A1

L13: Entry 2 of 412 File: PGPB Jul 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020099197
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020099197 A1

TITLE: NOVEL POTASSIUM CHANNEL MOLECULES AND USES THEREFOR

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 20020082224 A1

L13: Entry 3 of 412 File: PGPB Jun 27, 2002

PGPUB-DOCUMENT-NUMBER: 20020082224
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020082224 A1

TITLE: NON-IMMUNOGENIC PRODRUGS AND SELECTABLE MARKERS FOR USE IN GENE THERAPY

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 4. Document ID: US 20020064798 A1

L13: Entry 4 of 412 File: PGPB May 30, 2002

PGPUB-DOCUMENT-NUMBER: 20020064798
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020064798 A1

TITLE: COMBINATORIAL ENZYMATIC COMPLEXES

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 5. Document ID: US 20020058249 A1

L13: Entry 5 of 412 File: PGPB May 16, 2002

PGPUB-DOCUMENT-NUMBER: 20020058249
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020058249 A1

TITLE: DNA SHUFFLING TO PRODUCE HERBICIDE SELECTIVE CROPS

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 20020055474 A1

L13: Entry 6 of 412 File: PGPB May 9, 2002

PGPUB-DOCUMENT-NUMBER: 20020055474
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020055474 A1

TITLE: NOVEL MOLECULES OF THE TNF LIGAND SUPERFAMILY AND USES THEREFOR

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 7. Document ID: US 20020051971 A1

L13: Entry 7 of 412 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020051971
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020051971 A1

TITLE: USE OF MICROFLUIDIC SYSTEMS IN THE DETECTION OF TARGET

ANALYTES USING MICROSPHERE ARRAYS

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image KWIC

☐ 8. Document ID: US 20020048749 A1

L13: Entry 8 of 412 File: PGPB Apr 25, 2002

PGPUB-DOCUMENT-NUMBER: 20020048749
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020048749 A1

TITLE: METHODS FOR POLYMORPHISM IDENTIFCATION AND PROFILING

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image KWIC

☐ 9. Document ID: US 20020025551 A1

L13: Entry 9 of 412 File: PGPB Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025551
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025551 A1

TITLE: NOVEL MOLECULES OF THE T129-RELATED PROTEIN FAMILY AND USES THEREOF

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image KWIC

☐ 10. Document ID: US 20020025520 A1

L13: Entry 10 of 412 File: PGPB Feb 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020025520
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020025520 A1

TITLE: ITERATIVE RESEQUENCING

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image KWIC

Generate Collection Print

Terms	Documents
L12 and @ad<20000621	412

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Search Results - Record(s) 11 through 20 of 412 returned.

☐ 11. Document ID: US 20020019020 A1

L13: Entry 11 of 412 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019020
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019020 A1

TITLE: METHODS FOR TREATING CARDIOVASCULAR DISORDERS

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 12. Document ID: US 20020018774 A1

L13: Entry 12 of 412 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020018774
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020018774 A1

TITLE: CANDIDA ALBICANS mRNA 5'5-TRIPHOSPHATASE (CET-1) POLYNUCLEOTIDES

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 13. Document ID: US 20020012914 A1

L13: Entry 13 of 412 File: PGPB Jan 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020012914
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020012914 A1

TITLE: METHOD FOR TRANSFERRING NUCLEIC ACID INTO MULTICELLED EUKARYOTIC ORGANISM CELLS AND COMBINATION THEREFOR

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 14. Document ID: US 20020001830 A1

L13: Entry 14 of 412 File: PGPB Jan 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020001830
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020001830 A1

TITLE: SHUTTLE VECTORS

☐ 15. Document ID: US 20010051374 A1

L13: Entry 15 of 412 File: PGPB Dec 13, 2001

PGPUB-DOCUMENT-NUMBER: 20010051374
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010051374 A1

TITLE: HIGH EFFICIENCY GENETIC MODIFICATION METHODS

☐ 16. Document ID: US 20010051335 A1

L13: Entry 16 of 412 File: PGPB Dec 13, 2001

PGPUB-DOCUMENT-NUMBER: 20010051335
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010051335 A1

TITLE: POLYNUCLEOTIDES AND POLYPEPTIDES DERIVED FROM CORN TASSEL

☐ 17. Document ID: US 20010003042 A1

L13: Entry 17 of 412 File: PGPB Jun 7, 2001

PGPUB-DOCUMENT-NUMBER: 20010003042
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010003042 A1

TITLE: MULTIPARAMETER FACS ASSAYS TO DETECT ALTERATIONS IN CELL CYCLE REGULATION

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 18. Document ID: US 6436685 B1

L13: Entry 18 of 412 File: USPT Aug 20, 2002

US-PAT-NO: 6436685
DOCUMENT-IDENTIFIER: US 6436685 B1

TITLE: CSAPTP protein molecules and uses therefor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 19. Document ID: US 6432639 B1

L13: Entry 19 of 412 File: USPT Aug 13, 2002

US-PAT-NO: 6432639
DOCUMENT-IDENTIFIER: US 6432639 B1

TITLE: Isolated CYP3A4 nucleic acid molecules and detection methods

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

☐ 20. Document ID: US 6426186 B1

L13: Entry 20 of 412 File: USPT Jul 30, 2002

US-PAT-NO: 6426186
DOCUMENT-IDENTIFIER: US 6426186 B1

TITLE: Bone remodeling genes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image KWIC

Generate Collection Print

Terms	Documents
L12 and @ad<20000621	412

Display Format: - Change Format

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Search Results - Record(s) 21 through 30 of 412 returned.

☐ 21. Document ID: US 6423494 B1

L13: Entry 21 of 412 File: USPT Jul 23, 2002

US-PAT-NO: 6423494
DOCUMENT-IDENTIFIER: US 6423494 B1

TITLE: DR6 and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image | KWIC

☐ 22. Document ID: US 6413774 B1

L13: Entry 22 of 412 File: USPT Jul 2, 2002

US-PAT-NO: 6413774
DOCUMENT-IDENTIFIER: US 6413774 B1

TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image | KWIC

☐ 23. Document ID: US 6410328 B1

L13: Entry 23 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410328
DOCUMENT-IDENTIFIER: US 6410328 B1

TITLE: Sensitizing cells to compounds using lipid-mediated gene and compound delivery

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw. Desc | Image | KWIC

☐ 24. Document ID: US 6410301 B1

L13: Entry 24 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410301
DOCUMENT-IDENTIFIER: US 6410301 B1

TITLE: Myxococcus host cells for the production of epothilones

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 25. Document ID: US 6410241 B1

L13: Entry 25 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410241
DOCUMENT-IDENTIFIER: US 6410241 B1

TITLE: Methods of screening open reading frames to determine whether they encode polypeptides with an ability to generate an immune response

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 26. Document ID: US 6410240 B1

L13: Entry 26 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410240
DOCUMENT-IDENTIFIER: US 6410240 B1

TITLE: RGS-containing molecules and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 27. Document ID: US 6410232 B1

L13: Entry 27 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410232
DOCUMENT-IDENTIFIER: US 6410232 B1

TITLE: Molecules of the follistatin-related protein family and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 28. Document ID: US 6410220 B1

L13: Entry 28 of 412 File: USPT Jun 25, 2002

US-PAT-NO: 6410220
DOCUMENT-IDENTIFIER: US 6410220 B1

TITLE: Self-assembling genes, vectors and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 29. Document ID: US 6406884 B1

L13: Entry 29 of 412 File: USPT Jun 18, 2002

US-PAT-NO: 6406884
DOCUMENT-IDENTIFIER: US 6406884 B1

TITLE: Secreted proteins and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 30. Document ID: US 6403778 B1

L13: Entry 30 of 412 File: USPT Jun 11, 2002

US-PAT-NO: 6403778
DOCUMENT-IDENTIFIER: US 6403778 B1

TITLE: Toxicological response markers

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Terms	Documents
L12 and @ad<20000621	412

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=> d his

(FILE 'HOME' ENTERED AT 15:11:58 ON 24 AUG 2002)

FILE 'REGISTRY' ENTERED AT 15:12:03 ON 24 AUG 2002
L1 1 S CYTOCHROME P 450 REDUCTASE/CN

FILE 'HCAPLUS' ENTERED AT 15:12:47 ON 24 AUG 2002

FILE 'REGISTRY' ENTERED AT 15:12:54 ON 24 AUG 2002
SET SMARTSELECT ON
L2 SEL L1 1- CHEM : 11 TERMS
SET SMARTSELECT OFF

FILE 'HCAPLUS' ENTERED AT 15:12:54 ON 24 AUG 2002
L3 3283 S L2
L4 19819 S L3 OR CYTOCHROME P450
L5 2445 S L4 (L) (HUMAN OR MAN)
L6 46 S L5 (L) (NUCLEIC ACID OR POLYNUCLEOTIDE OR NUCLEOTIDE OR VECTO
L7 40 S L6 AND PD<20000621

L2 ANSWER 603 OF 603 REGISTRY COPYRIGHT 2002 ACS
RN 9039-06-9 REGISTRY
CN Reductase, cytochrome P 450 (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Cytochrome P 450 reductase
CN **Cytochrome P450 NADPH reductase**
CN Dihydronicotinamide adeninedinucleotide phosphate-cytochrome P 450 reductase
CN Ferrihemoprotein P 450 reductase
CN NADH-cytochrome P 450 reductase
CN NADPH-cytochrome P 450 reductase
CN NADPH-dependent tocopherolquinone reductase
CN Reduced nicotinamide adenine dinucleotide phosphate-cytochrome P 450 reductase
CN TPNH-cytochrome P 450 reductase
DR 9046-76-8
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CHEMCATS, EMBASE, PROMT, TOXCENTER, USPATFULL

=> d ibib ab 1-40

L7 ANSWER 1 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:771048 HCAPLUS
DOCUMENT NUMBER: 135:328149
TITLE: Protein and cDNA sequences of human, mouse, and Danio rerio retinoic acid-metabolizing protein, and uses thereof
INVENTOR(S): Petkovich, P. Martin; White, Jay A.; Beckett, Barbara R.; Jones, Glenville
PATENT ASSIGNEE(S): Queen's University at Kingston, Can.
SOURCE: U.S., 75 pp., Cont.-in-part of Appl. No. PCT/CA97/00440.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6306624	B1	20011023	US 1997-882164	19970625
US 6063606	A	20000516	US 1996-724466	19961001 <--
WO 9749815	A1	19971231	WO 1997-CA440	19970623 <--

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1996-667546 B2 19960621
US 1996-724466 A2 19961001
WO 1997-CA440 A2 19970623

AB This invention provides protein and cDNA sequences of human, mouse, and zebrafish (Danio rerio) retinoic acid-metabolizing protein. The protein is shown to have the ability to hydroxylate retinoic acid (RA) at the 4 position of the .beta.-ionone ring. The protein of the invention belongs to the family of cytochrome P450s, and its prodn. in epithelial cells is induced by treatment with RA. The invention also relates to the use of the provided proteins and cDNAs, particularly in drug screening assays. The examples disclose studies of the effects of 4-Hydroxyphenylretinamide, RA, ketoconazole, and Am580 upon the expression of the protein.
REFERENCE COUNT: 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:858190 HCAPLUS
DOCUMENT NUMBER: 135:147884
TITLE: Use of single nucleotide polymorphisms (SNP) and real-time polymerase chain reaction for bone marrow engraftment analysis
AUTHOR(S): Oliver, Dwight H.; Thompson, Richard E.; Griffin, Constance A.; Eshleman, James R.
CORPORATE SOURCE: Division of Molecular Pathology of the Department of Pathology, Johns Hopkins Hospital, Baltimore, MD, 21205, USA
SOURCE: Journal of Molecular Diagnostics (**2000**), 2(4), 202-208
CODEN: JMDIFP; ISSN: 1525-1578
PUBLISHER: Association for Molecular Pathology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Allogeneic bone marrow transplant engraftment assays use polymorphisms in the **human** genome to det. the relative percentages of donor and recipient cells present in the recipient. We describe a novel

posttransplant assay approach using single **nucleotide** polymorphisms (SNPs), the most common type of polymorphism in **humans**. Using samples of defined genotype, we used real-time polymerase chain reaction (PCR) and allele-specific fluorescent TaqMan probes to assay a SNP of the **cytochrome P450** CYP2C9 gene. Std. curves of chimeric mixes showed a linear relationship between the ratio of two alleles and the ratio of their resp. fluorophore emission, except for mixes with a low percentage (<5%) of the less common allele. We validated the SNP real-time PCR assay by comaring it to Southern hybridization anal., analyzing DNA mixes in a blinded fashion with both methods. The correlation between the two methods was high. We have produced a statistical model that varies allele frequency to predict how many SNPs would be required to produce a functional SNP panel. Addnl. development will be necessary to produce such a panel of highly informative SNPs for clin. use. A real-time PCR SNP assay may ultimately provide more accurate quantification and shortened turnaround time compared to current post-engraftment assays.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:522251 HCAPLUS

DOCUMENT NUMBER: 133:202548

TITLE: Phenytoin metabolism by human cytochrome P450: involvement of P450 3A and 2C forms in secondary metabolism and drug-protein adduct formation

AUTHOR(S): Cuttle, Leila; Munns, Andrew J.; Hogg, Nicole A.; Scott, Justin R.; Hooper, Wayne D.; Dickinson, Ronald G.; Gillam, Elizabeth M. J.

CORPORATE SOURCE: Department of Physiology and Pharmacology, University of Queensland, St. Lucia, 4072, Australia

SOURCE: Drug Metabolism and Disposition (**2000**), 28(8), 945-950
CODEN: DMDSAI; ISSN: 0090-9556

PUBLISHER: American Society for Pharmacology and Experimental Therapeutics

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The anticonvulsant phenytoin (5,5-diphenylhydantoin) provokes a skin rash in 5 to 10% of patients, which heralds the start of an idiosyncratic reaction that may result from covalent modification of normal self proteins by reactive drug metabolites. Phenytoin is metabolized by cytochrome P 450 (P 450) enzymes primarily to 5-(p-hydroxyphenyl-),5-phenylhydantoin (HPPH), which may be further metabolized to a catechol that spontaneously oxidizes to semiquinone and quinone species that covalently modify proteins. The aim of this study was to det. which P450s catalyze HPPH metab. to the catechol, proposed to be the final enzymic step in phenytoin bioactivation. Recombinant **human** P450s were coexpressed with **NADPH-cytochrome P 450 reductase** in Escherichia coli. Novel bicistronic expression **vectors** were constructed for P 450 2C19 and the three major variants of P 450 2C9, i.e., 2C9*1, 2C9*2, and 2C9*3. HPPH metab. and covalent adduct formation were assessed in parallel. P 450 2C19 was the most effective catalyst of HPPH oxidn. to the catechol metabolite and was also assocd. with the highest levels of covalent adduct formation. P 450 3A4, 3A5, 3A7, 2C9*1, and 2C9*2 also catalyzed bioactivation of HPPH, but to a lesser extent. Fluorog. anal. showed that the major targets of adduct formation in bacterial membranes were the catalytic P 450 forms, as suggested from expts. with **human** liver microsomes. These results suggest that P 450 2C19 and other forms from the 2C and 3A subfamilies may be targets as well as catalysts of drug-protein adduct formation from phenytoin.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:521467 HCAPLUS

DOCUMENT NUMBER: 133:188406
TITLE: Identification of a 25-hydroxyvitamin D3 1.alpha.-hydroxylase gene transcription product in cultures of human syncytiotrophoblast cells
AUTHOR(S): Diaz, Lorenza; Sanchez, Irene; Avila, Euclides; Halhali, Ali; Vilchis, Felipe; Larrea, Fernando
CORPORATE SOURCE: Department of Reproductive Biology, Instituto Nacional de la Nutricion Salvador Zubiran, Mexico City, 14000, Mex.
SOURCE: Journal of Clinical Endocrinology and Metabolism (**2000**), 85(7), 2543-2549
CODEN: JCEMAZ; ISSN: 0021-972X
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although accumulating data show that placenta is able to synthesize 1,25-dihydroxyvitamin D3, the presence of **cytochrome P450** enzyme capable of converting 25-hydroxyvitamin D3 (25OHD3) to the biol. active form of vitamin D in this tissue, has not been yet clearly established. In this study, we have investigated the presence of 25-hydroxyvitamin D3 1.alpha.-hydroxylase (1.alpha.-(OH)ase) gene expression products in cultured **human** syncytiotrophoblast. Total RNA was isolated from cultured placental cells and subjected to Northern blots or RT-PCR by using 1.alpha.-(OH)ase-specific primers. The amplified complementary DNA fragments were analyzed by gel electrophoresis and **nucleotide** sequencing. Total RNA from kidney HEK 293 cells was subjected to reverse transcriptase reaction, and a 298-bp complementary DNA 1.alpha.-(OH)ase probe was generated by PCR. Primary cultures of **human** syncytiotrophoblasts exhibited 1.alpha.-(OH)ase activity, and a transcript for this gene could be demonstrated in these cells. Northern blot anal. revealed the presence of a 2.5-kb product, similar in size to that previously reported in kidney. RT-PCR anal. demonstrated the presence of a single transcript with **nucleotide** sequence identical to that previously reported for **human** 1.alpha.-(OH)ase complementary DNA clones. In addn., data are presented which suggest that differentiation of cytotrophoblast to the syncytial state was not necessary for this gene to be expressed, which may indicate a role of this enzyme all through pregnancy. The overall results of this study provide evidence for the presence of 1.alpha.-(OH)ase in the **human** placenta, suggesting that conversion of 25OHD3 to 1,25-dihydroxyvitamin D3 in the trophoblast is most probably attributed to an enzymic 1.alpha.-hydroxylation reaction.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:459322 HCAPLUS
DOCUMENT NUMBER: 133:204607
TITLE: Metabolic activation of N-alkylnitrosamines in genetically engineered Salmonella typhimurium expressing CYP2E1 or CYP2A6 together with human NADPH-cytochrome P450 reductase
AUTHOR(S): Kushida, Hirotaka; Fujita, Ken-Ichi; Suzuki, Akihiro; Yamada, Masami; Endo, Toru; Nohmi, Takehiko; Kamataki, Tetsuya
CORPORATE SOURCE: Laboratory of Drug Metabolism, Division of Pharmacobio-dynamics, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060-0812, Japan
SOURCE: Carcinogenesis (**2000**), 21(6), 1227-1232
CODEN: CRNGDP; ISSN: 0143-3334
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A Salmonella typhimurium tester strain YG7108 2E1/OR co-expressing human CYP2E1 together with human NADPH-cytochrome P 450 reductase (OR) was established. The mutagen-activating capacity of human CYP2E1 for

N-alkylnitrosamines was compared with that of CYP2A6 using the YG7108 2E1/OR and the YG7108 2A6/OR strains of Salmonella. Salmonella YG7108 2A6/OR is a deriv. of YG7108 co-expressing CYP2A6 together with OR. Eight N-alkylnitrosamines, including N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosomethylphenylamine (NMPhA), N-nitrosopyrrolidine (NPYR), N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) were examd. CYP2E1 expressed in the YG7108 2E1/OR cells showed mutagen-activating capacity, as indicated by induced revertants/min/pmol cytochrome P 450, for NDMA, NDEA, NDPA, NDBA, NPYR and NNK, but not NMPhA and NNN. CYP2A6 activated NDMA, NDEA, NDPA, NDBA, NMPhA, NPYR, NNN and NNK. The ratio of the mutagen-activating capacity seen with CYP2A6 to that seen with CYP2E1 was calcd. for each N-alkylnitrosamine. In the case of NDMA, NPYR and NDEA, the ratio was under 1.0, while the ratio was over 1.0 with NDPA, NDBA, NNK, NMPhA and NNN. We conclude that human CYP2E1 is mainly responsible for the metabolic activation of N-nitrosamines with a relatively short alkyl chain(s), whereas CYP2A6 was predominantly responsible for the metabolic activation of N-alkylnitrosamines possessing a relatively bulky alkyl chain(s).

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:398552 HCAPLUS
DOCUMENT NUMBER: 133:99789
TITLE: Conversion of pregnenolone to DHEA by human 17.alpha.-hydroxylase/17,20-lyase (P450c17). Evidence that DHEA is produced from the released intermediate, 17.alpha.-hydroxypregnenolone
AUTHOR(S): Soucy, Penny; Luu-The, Van
CORPORATE SOURCE: Medical Research Council Group in Molecular Endocrinology, Oncology and Molecular Endocrinology Research Center, CHUQ CHUL and Laval University, Ste-Foy, QC, G1V 4G2, Can.
SOURCE: European Journal of Biochemistry (**2000**), 267(11), 3243-3247
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Most previous studies using reconstituted systems and fast kinetics suggest that the conversion of pregnenolone to dehydroepiandrosterone (DHEA; the precursor of androgen and estrogen biosynthesis) by P 450c17 does not require the release of the intermediate 17.alpha.-OHPreg (a precursor of cortisol biosynthesis). With such a mechanism, it is difficult to conceive how high amts. of DHEA may be produced in some cells or tissues, such as the testis and cells from the adrenal reticularis, while in other tissues such as the fasciculata zone, high levels of 17.alpha.-OHPreg are synthesized. The authors address this matter using intact transfected cells, which better reflect the actual cellular conditions. Furthermore, by using transfected cells, the authors can conveniently analyze **human** enzymes, as they are not restricted by the availability of **human** tissues as in the case of methods using purified or partially purified enzymes. Using intact HEK-293 cells transfected with **human** P 450c17 in culture, the authors showed, in a time course study of the transformation of pregnenolone, that there is an accumulation of 17.alpha.-OHPreg, and that, subsequently, the accumulated 17.alpha.-OHPreg decreases with a concomitant increase in DHEA prodn. The DHEA/17.alpha.-OHPreg ratio changes from 0.1:1 after 1 h incubation to 50: 1 after 20 h. This result strongly suggests that the transformation of Preg to DHEA proceeds through two steps in which DHEA is produced from the released intermediate 17.alpha.-OHPreg. The authors also show that high levels of substrate vs. enzyme concn. will lead to high hydroxylase activity, whereas the reverse will increase the lyase activity. The result is in good agreement with recent observations suggesting that surrounding enzymes and steroids could modulate the lyase

activity. Contransfection of **vectors** expressing cytochrome b5 and **NADPH cytochrome P 450 reductase** indicates that both are required for an optimum prodn. of DHEA.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:175952 HCAPLUS
DOCUMENT NUMBER: 132:231943
TITLE: Gene-directed enzyme prodrug therapy for treatment of cancer using a replication-deficient adenovirus type 5 expression vector
INVENTOR(S): Steiner, Mitchell S.; Lu, Yi
PATENT ASSIGNEE(S): Genotherapeutics, Inc., USA
SOURCE: PCT Int. Appl., 110 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000014256	A1	20000316	WO 1999-US18834	19990903 <--

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9957783	A1	20000327	AU 1999-57783	19990903 <--

PRIORITY APPLN. INFO.: US 1998-148275 A 19980904
WO 1999-US18834 W 19990903

AB Gene-directed enzyme prodrug therapy (GDEPT) for treatment of cancer using a replication-deficient adenovirus type 5 expression **vector** is described. This invention provides a replication-deficient adenovirus type 5 expression **vector** with a deletion in the E1 and E3 region of the adenovirus genome and an insertion of a **nucleic acid** encoding a cytochrome 2C9 P 450 (CYP2C9), cytochrome 3A4 P 450 (CYP3A4), or **NADPH cytochrome P 450 reductase** under the control of a Rous Sarcoma Virus promoter. This invention provides a method of inducing chemotoxicity in a tumor cell and treating a subject with cancer by transfecting the tumor cell with the replication-deficient adenovirus type 5 expression **vectors** and thereafter administering a prodrug, whereby the prodrug is activated selectively in tumor cells transfected with the adenovirus **vector** and expressing the prodrug activating enzymes. Cyclophosphamide (CPA), infosamide, or methylpropylchloronitrosourea can be used as prodrugs. PPC-1 prostate cancer cell line transfected with a replication-deficient adenovirus type 5 expression **vector** having a CYP2C9 encoding gene overexpressed CYP2C9, and was sensitized to CPA chemotoxicity. The same was true for 9L rat glioma cells and DU145 prostate cancer cells. CYP2C9 gene had a stronger activation of CPA than CYP3A4 gene. In addn., a bystander effect was obsd. where **human** lymphoblastoma cell line H2C9 expressing CYP2C9 sensitized adjacent CYP2C9 neg. PPC-1 cells. Also, coexpression of **NADPH cytochrome P 450 reductase** argumented the effect of CYP2C9 on CPA-induced cytotoxicity in PPC-1 cells.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:577033 HCAPLUS
DOCUMENT NUMBER: 131:194269
TITLE: Prodrug activating agent comprising localization domain-prodrug activation domain fusions and hematopoietic cells producing them for use as pharmaceuticals
INVENTOR(S): Stratford, Ian James; Patterson, Adam Vorn; Kingsman, Susan Mary; Kan, On; Griffiths, Leigh; Mitrophanous, Kyriacos
PATENT ASSIGNEE(S): Oxford Biomedica (Uk) Ltd., UK
SOURCE: PCT Int. Appl., 190 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9945127	A2	19990910	WO 1999-GB674	19990305 <--
WO 9945127	A3	20000224		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2322664	AA	19990910	CA 1999-2322664	19990305 <--
AU 9932670	A1	19990920	AU 1999-32670	19990305 <--
EP 1068338	A2	20010117	EP 1999-937944	19990305
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:

GB 1998-4841	A	19980306
GB 1998-18103	A	19980819
GB 1999-2081	A	19990129
WO 1999-GB674	W	19990305

AB A prodrug activating agent comprising: (a) a localization domain and (b) a prodrug activation domain for activating a prodrug in a target cell, **nucleic acids** and **vectors** encoding these agents, hematopoietic stem cells expressing the **nucleic acid**, and pharmaceutical compns. contg. said agents or **nucleic acids** are disclosed. Chimeric genes for numerous prodrug activating agents were prepd. One such gene encoded a fusion of SV40 large T antigen nuclear localization signal fused to a **human cytochrome P 450 reductase** fragment comprising the FAD- and NADH-binding domains. Equine infectious anemia virus **vectors** for expression of such chimeric genes were also prepd. When macrophages infected with adenovirus contg. a CMV promoter fused to **human** cytochrome P 450-2B6 cDNA were incubated with tumor cells in the presence of cyclophosphamide, the tumor cells were killed. Under the same conditions, tumor cells in the presence of unmodified macrophages and cyclophosphamide were not killed.

L7 ANSWER 9 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:215790 HCAPLUS
DOCUMENT NUMBER: 131:53665
TITLE: The flavonoid galangin is an inhibitor of CYP1A1 activity and an agonist/antagonist of the aryl hydrocarbon receptor
AUTHOR(S): Ciolino, H. P.; Yeh, G. C.
CORPORATE SOURCE: Cellular Defense and Carcinogenesis Section, Basic Research Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201, USA

SOURCE: British Journal of Cancer (**1999**), 79(9/10), 1340-1346
CODEN: BJCAAI; ISSN: 0007-0920
PUBLISHER: Churchill Livingstone
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effect of the dietary flavonoid galangin on the metab. of 7,12-dimethylbenz[a]anthracene (DMBA), the activity of **cytochrome P450** 1A1 (CYP1A1), and the expression of CYP1A1 in MCF-7 **human** breast carcinoma cells was investigated. Galangin inhibited the catabolic breakdown of DMBA, as measured by thin-layer chromatog., in a dose-dependent manner. Galangin also inhibited the formation of DMBA-DNA adducts, and prevented DMBA-induced inhibition of cell growth. Galangin caused a potent, dose-dependent inhibition of CYP1A1 activity, as measured by ethoxyresorufin-O-deethylase activity, in intact cells and in microsomes isolated from DMBA-treated cells. Anal. of the inhibition kinetics by double-reciprocal plot demonstrated that galangin inhibited CYP1A1 activity in a non-competitive manner. Galangin caused an increase in the level of CYP1A1 mRNA, indicating that it may be an agonist of the aryl hydrocarbon receptor, but it inhibited the induction of CYP1A1 mRNA by DMBA or by 2,3,5,7-tetrachlorodibenzo-p-dioxin (TCDD). Galangin also inhibited the DMBA- or TCDD-induced transcription of a reporter **vector** contg. the CYP1A1 promoter. Thus, galangin is a potent inhibitor of DMBA metab. and an agonist/antagonist of the AhR, and may prove to be an effective chemopreventive agent.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:96379 HCAPLUS
DOCUMENT NUMBER: 130:163172
TITLE: Methods of using cytochrome P450 reductase for the enhancement of P450-based anticancer gene therapy
INVENTOR(S): Waxman, David J.; Chen, Ling
PATENT ASSIGNEE(S): Trustees of Boston University, USA
SOURCE: PCT Int. Appl., 135 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9905299	A1	19990204	WO 1998-US15302	19980723 <--
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6207648	B1	20010327	US 1998-118179	19980717
AU 9887578	A1	19990216	AU 1998-87578	19980723 <--
EP 1017835	A1	20000712	EP 1998-939083	19980723
R: DE, DK, ES, FR, GB, IT				

PRIORITY APPLN. INFO.:
US 1997-53677P P 19970724
US 1998-118179 A 19980717
WO 1998-US15302 W 19980723

AB Methods of killing neoplastic cells are provided. The invention relates to the use of NADPH-cytochrome P 450 reductase (RED) gene transfer in combination with cytochrome P 450 gene transfer to enhance the sensitivity of tumor cells to anticancer drugs that are activated by P 450 enzymes. The use of bioreductive drugs that are activated by RED and/or cytochrome P 450, in this paradigm, is also provided.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 11 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:238474 HCAPLUS
DOCUMENT NUMBER: 129:66474

TITLE: The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription

AUTHOR(S): Stratakis, Constantine A.; Vottero, Alessandra; Brodie, Angela; Kirschner, Lawrence S.; Deatkine, David; Lu, Qing; Yue, Wei; Mitsiades, Constantine S.; Flor, Armando W.; Chrousos, George P.

CORPORATE SOURCE: Section on Pediatric Endocrinology, Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892, USA

SOURCE: Journal of Clinical Endocrinology and Metabolism (**1998**), 83(4), 1348-1357
CODEN: JCEMAZ; ISSN: 0021-972X

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Increased extraglandular aromatization has been reported as the cause of familial gynecomastia. The authors studied a kindred with aromatase excess inherited in an autosomal dominant manner, in which affected males had heterosexual precocity and/or gynecomastia, and affected females had isosexual precocity and/or macromastia. The propositus was a 9-yr-old boy with gynecomastia. His 7.5-yr-old sister had precocious puberty, and their father and paternal grandmother had peripubertal gynecomastia and macromastia, resp. Serum concns. of gonadal and adrenal steroid hormones were detd. before and after the administration of corticotropin and/or hCG. Aromatase activity was detd. by [3H].DELTA.4-androstenedione to [3H]estrone conversion by cultured skin fibroblasts and/or Epstein-Barr virus-transformed lymphocytes and was detected by immunohistochem. and/or Western anal. Linkage was examd. with a polymorphism of the aromatase (P450arom) gene. The P450arom mRNA was analyzed by rapid amplification of complementary DNA (cDNA) ends, RNase protection assay, and RT-PCR. HCG testing demonstrated a high rate of conversion of .DELTA.4-androstenedione to estrone and of testosterone to estradiol in the propositus and his father. Treatment of the propositus and his sister was initiated with an aromatase inhibitor (testolactone) and a GnRH analog, which successfully delayed skeletal and pubertal development in both children. Markedly increased aromatase activity was found in the patients' fibroblasts and Epstein-Barr virus-transformed lymphocytes. The P450arom polymorphism segregated with the disease in the family. A new 5'-splice variant was present in the patients' P450arom mRNA, thus identifying yet another first exon of this gene, which appears to be aberrantly expressed in this family. In conclusion, a family with the aromatase excess syndrome is described, in which the condition was inherited in an autosomal dominant manner, led to feminizing manifestations in both sexes, and was assocd. with the aberrant utilization of a novel transcript of the P450arom gene.

L7 ANSWER 12 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:168689 HCAPLUS

DOCUMENT NUMBER: 128:292136

TITLE: Functional co-expression of CYP2D6 and human NADPH-cytochrome P450 reductase in Escherichia coli

AUTHOR(S): Pritchard, Michael P.; Glancey, Michael J.; Blake, Jennifer A. R.; Gilham, David E.; Burchell, Brian; Wolf, C. Roland; Friedberg, Thomas

CORPORATE SOURCE: Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK

SOURCE: Pharmacogenetics (**1998**), 8(1), 33-42
CODEN: PHMCEE; ISSN: 0960-314X

PUBLISHER: Chapman & Hall

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The polymorphic human CYP2D6 has been co-expressed with human NADPH-cytochrome P 450 oxidoreductase in Escherichia coli in order to generate a functional recombinant monooxygenase system for the study of

xenobiotic metab. The two cDNAs were co-expressed from sep., compatible plasmids with different antibiotic selection markers. The CYP2D6 could be detected in bacterial cells at levels up to 700 nmol l-1 culture by Fe2+ - CO vs. Fe2+ difference spectroscopy, exhibiting the characteristic absorbance peak at 450 nm. Immunoblotting demonstrated the presence of both proteins in bacterial membranes, where they were expressed at levels significantly higher than those found in human liver microsomes. Membrane content was 150-200 pmol CYP2D6 (detd. spectrally) and 100-230 pmol CYP-reductase (detd. enzymically) per mg protein. Critically, the two co-expressed proteins were able to couple to form a NADPH-dependent monooxygenase which metabolized the CYP2D6 substrate bufuralol (Vmax 3.30 nmol min-1 mg-1 protein; Km 11.1 .mu.M) in isolated membrane fractions. This Km value was similar to the Km detd. in human liver microsomes. Activity could be inhibited by the specific inhibitor quinidine. Of greater significance however, was the finding that intact E. coli cells, even in the absence of exogenous NADPH, were able to metabolize bufuralol at rates almost as high as those measured in membranes (4.6 .+-. 0.4 min-1 vs. 5.7 .+-. 0.2 min-1 at 50 .mu.M substrate). Such recombinant strains will greatly facilitate the mol. characterization of allelic variants of cytochrome P 450 isoenzymes.

L7 ANSWER 13 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:74060 HCAPLUS
DOCUMENT NUMBER: 128:111790
TITLE: Metabolic Activation of Aromatic Amine Mutagens by Simultaneous Expression of Human Cytochrome P450 1A2, NADPH-Cytochrome P450 Reductase, and N-Acetyltransferase in Escherichia Coli
AUTHOR(S): Josephy, P. David; Evans, David H.; Parikh, Asit; Guengerich, F. Peter
CORPORATE SOURCE: Guelph-Waterloo Centre for Graduate Work in Chemistry, Departments of Chemistry and Biochemistry and of Molecular Biology and Genetics, University of Guelph, ON, N1G 2W1, Can.
SOURCE: Chemical Research in Toxicology (**1998**), 11(1), 70-74
CODEN: CRTOEC; ISSN: 0893-228X
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors describe the construction of a new strain of Escherichia coli designed to bioactivate arom. amines and to detect their mutagenicity with high sensitivity. Strain DJ4309 bears two plasmids, a pACYC184-derived plasmid which expresses Salmonella typhimurium acetyl CoA:arylamine N-acetyltransferase (NAT) and a pBR322-derived plasmid which expresses **human** cytochrome P 450 1A2 and **NADPH-cytochrome P 450 reductase**. The combined actions of these enzymes convert arom. amines into reactive, mutagenic N-acetoxy esters. The strain also carries a mutated copy of the lacZ gene (on an F' factor) which reverts to the wild-type gene by a -(GpC) frameshift mutation. Strain DJ4309 expresses high levels of NAT and cytochrome P 450 1A2 and is very sensitive to mutagenesis induced by representative arom. amines. Mutagenicity of 2-aminoanthracene in strain DJ4309 is higher than what can be obtained by rat liver homogenate 9000g supernatant (S9) activation in the parent strain lacking the P 450 expression **vector**. Strain DJ4309 provides a useful system for detecting mutagenic arom. amines and for studying their metab. by **human** P 450 1A2.

L7 ANSWER 14 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:42508 HCAPLUS
DOCUMENT NUMBER: 128:111546
TITLE: Method of identifying cytochrome P450-encoding nucleic acid
INVENTOR(S): Petkovich, P. Martin
PATENT ASSIGNEE(S): Queen's University At Kingston, Can.; Petkovich, P. Martin

SOURCE: PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9749832	A2	19971231	WO 1997-CA488	19970623 <--
WO 9749832	A3	19980326		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 6063606	A	20000516	US 1996-724466	19961001 <--
AU 9733321	A1	19980114	AU 1997-33321	19970623 <--
EP 935676	A2	19990818	EP 1997-929069	19970623 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000513928	T2	20001024	JP 1998-502013	19970623

PRIORITY APPLN. INFO.:
US 1996-667546 A2 19960621
US 1996-724466 A2 19961001
WO 1997-CA488 W 19970623

AB A method for identifying a nucleotide sequence encoding a cytochrome P 450, the cytochrome being inducible or suppressible in cells of a given type by an agent is disclosed. The method includes: exposing a first group of the cells to the agent so as to induce or suppress expression of the cytochrome P 450; isolating first mRNA from the cells; isolating second mRNA from a second group of the cells which have not been exposed to the agent so as to induce or suppress expression of the cytochrome P 450; amplifying the first and second mRNA, resp., in the presence of an oligo(dT)-based first nucleic acid primer sufficient to prime synthesis from a poly(A) tail and a second nucleic acid primer substantially complementary to a nucleic acid sequence encoding a conserved region of a known cytochrome P 450; displaying amplified products of the first mRNA and amplified products of the second mRNA to detect differences therebetween; and identifying said nucleotide sequence encoding said inducible or suppressible cytochrome P 450. CDNA for all-trans-retinol-induced zebrafish cytochrome P450RAI mRNA was isolated using the above method. The human homolog of this cDNA as well as the promoters for the human, mouse and zebrafish cytochrome P450RAI were also prepd., sequenced and characterized.

L7 ANSWER 15 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:642129 HCAPLUS
DOCUMENT NUMBER: 127:327506
TITLE: Expression of cytochrome P450 3A7 in Escherichia coli: effects of 5' modification and catalytic characterization of recombinant enzyme expressed in bicistronic format with NADPH-cytochrome P450 reductase
AUTHOR(S): Gillam, Elizabeth M. J.; Wunsch, Rebecca M.; Ueng, Yune-Fang; Shimada, Tsutomu; Reilly, Paul E. B.; Kamataki, Tetsuya; Guengerich, F. Peter
CORPORATE SOURCE: Dep. Physiol. Pharmacol., Univ. Queensland, St. Lucia, 4072, Australia
SOURCE: Archives of Biochemistry and Biophysics (**1997**), 346(1), 81-90
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cytochrome P 450 3A7 is the major P 450 form present in fetal liver tissue and may be responsible for the detoxification of many drugs that reach the fetal circulation. We report the development of bacterial expression systems for P 450 3A7. Maximal yields (up to 50 nmol P 450/L culture) were obtained with a construct in which the 5'-terminus of the 3A7 cDNA was modified to include the MALLLAVFL N-terminal sequence of recombinant bovine P 450 17A and to incorporate several downstream amino acid substitutions derived from the P 450 3A5 sequence. This sequence also appeared optimal for expression of P 450 3A4 and 3A5. Recombinant P 450 3A7 was partially purified using ion-exchange and hydroxylapatite chromatog. and reconstituted with **NADPH-cytochrome P 450 reductase**, cytochrome b5, and lipids. Activity comparable to that of P 450 3A4 was demonstrated toward a no. of procarcinogens. An alternative approach was used to further characterize recombinant 3A7 due to low yields of recombinant protein in the expression and poor recovery in the purifn. P 450 3A7 was subcloned into a bicistronic **vector** contg. **human NADPH-cytochrome P 450 reductase** and expressed in bacteria. Recombinant P 450 3A7 coexpressed in bacterial membranes with **NADPH-cytochrome P 450 reductase** showed similar levels of activity toward erythromycin (N-demethylatio) and ethylmorphine (N-demethylation) to P 450 3A4 and 3A5 expressed in the same system, whereas 3A7 was less active toward midazolam (1'-and 4-hydroxylation) and nifedipine (oxidn.).

L7 ANSWER 16 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	1996:388445 HCAPLUS
DOCUMENT NUMBER:	125:52167
TITLE:	Active-site structure analysis of recombinant human inducible nitric oxide synthase using imidazole
AUTHOR(S):	Chabin, Renee M.; McCauley, Ermenegilda; Calaycay, Jimmy R.; Kelly, Theresa M.; MacNaul, Karen L.; Wolfe, Gloria C.; Hutchinson, Nancy I.; Madhusudanaraju, Sayyaparaju; Schmidt, John A.; et al.
CORPORATE SOURCE:	Department of Biochemistry, Merck Research Laboratories, Rahway, NJ, 07065, USA
SOURCE:	Biochemistry (**1996**), 35(29), 9567-9575 CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER:	American Chemical Society
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Nitric oxide synthase catalyzes the pyridine **nucleotide** -dependent oxidn. of L-arginine to nitric oxide and L-citrulline. It is a specialized **cytochrome P450** monooxygenase that is sensitive to inhibition by imidazole. Steady-state kinetic studies on recombinant **human** inducible nitric oxide synthase (rH-iNOS) demonstrate that imidazole and 1-phenylimidazole are competitive and reversible inhibitors vs. L-arginine. Structure-activity relationship and pH dependence studies on the inhibition suggest that the neutral form of imidazole may be the preferred species and that the only modifications allowed without the loss of inhibition are at the N-1 position of imidazole. Optical spectrophotometric studies of rH-iNOS with imidazole and 1-phenylimidazole yielded type II difference spectra exhibiting Kd values of 63 and 28 .mu.M, resp. These values were in good agreement with the steady-state Ki of 95 and 38 .mu.M, resp., and confirms the site of binding is at the sixth axial ligand of the heme. Imidazole (2.2 mM) also perturbed the Kd of L-arginine from 3.03 to 209 .mu.M. The obsd. increase in the Kd for L-arginine is consistent with imidazole being a competitive inhibitor vs. L-arginine. The IC50 values of imidazole and 1-phenylimidazole were lower in the absence of exogenous BH4, and both inhibitors also competitively inhibited the BH4-dependent activation of the enzyme. These data taken together suggest that the L-arginine, dioxygen, and the BH4 binding sites are in close proximity in rH-iNOS. Furthermore, these studies demonstrate the usefulness of imidazole compds. as active site probes for **human** recombinant **human** iNOS.

L7 ANSWER 17 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:162028 HCAPLUS
DOCUMENT NUMBER: 124:222067
TITLE: Coexpression of mammalian cytochrome P450 and reductase in Escherichia coli
AUTHOR(S): Dong, Jinsheng; Porter, Todd D.
CORPORATE SOURCE: College of Pharmacy, Univ. Kentucky, Lexington, KY, 40536-0082, USA
SOURCE: Archives of Biochemistry and Biophysics (**1996**), 327(2), 254-9
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB CDNAs for **human** cytochrome P 450 2E1 and rat **NADPH-cytochrome-P 450 reductase** were cloned sep. and in tandem into bacterial expression **vectors**, and expression of the two proteins in Escherichia coli was monitored by immunoblotting, spectroscopy, and catalytic assays. The cDNAs were sepd. on the coexpression plasmid by 22 **nucleotides**, with the P 450 cDNA preceding the reductase cDNA. P 450 content in solubilized cell membranes, whether expressed alone or coexpressed with P 450 reductase, was approx. 0.11 nmol/mg of protein, and approx. 0.8 nmol could be obtained per L of culture. Reductase content was five- to sixfold greater than P 450 content when coexpressed, but severalfold less than that obtained when expressed without the upstream P 450 cDNA, indicating differences in both stability and translatability between the two proteins. Solubilized membranes from cells expressing both proteins catalyzed aniline hydroxylation, p-nitrophenol hydroxylation, and N-nitrosodimethylamine demethylation at rates equiv. to those obtained by combining P 450 and reductase prepns.; addn. of purified reductase to these membranes did not augment the activity. However, in contrast to results obtained with P 450 2E1 expressed in other heterologous systems, addn. of rabbit liver cytochrome b5 to prepns. catalyzing p-nitrophenol or N-nitrosodimethylamine oxidn. did not increase turnover, and, although activity could be shown with unsolubilized membranes, oxidn. of these substrates in vivo could not be demonstrated. Nonetheless, the ability to coexpress P 450 and reductase in E. coli to generate a functional monooxygenase system in vitro enhances the utility of this organism for the expression and characterization of cloned P 450 isoforms.

L7 ANSWER 18 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:808950 HCAPLUS
DOCUMENT NUMBER: 124:80223
TITLE: Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform
AUTHOR(S): Zeldin, Darryl C.; DuBois, Raymond N.; Ralck, John R.; Capdevila, Jorge H.
CORPORATE SOURCE: Dep. Med., Vanderbilt Univ. Med. Sch., Nashville, TN, 37232, USA
SOURCE: Archives of Biochemistry and Biophysics (**1995**), 322(1), 76-86
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cDNA contg. an open reading frame coding for a **human** cytochrome P 450 arachidonic acid epoxygenase was isolated from a male **human** kidney cDNA library. Sequence anal. showed that, with few exceptions, this cDNA was nearly identical to the published sequence for **human** liver Cyp 2C8 (S. T. Okino et al., 1987, J. Biol. Chem. 262, 16072-16079) and encoded a polypeptide of 490 amino acids. **Nucleic acid** hybridization indicated that: (a) Cyp 2C8 and 2C10 were expressed at comparable levels in the **human** liver and (b) compared to Cyp 2C10, the steady state concns. of Cyp 2C8 transcripts in the **human** kidney were substantially lower. The

kidney 2C8 cDNA was cloned into a pBlue BacIII **vector**, expressed using a baculovirus/Sf9 insect cell system, and the recombinant Cyp 2C8 protein was purified by a combination of hydrophobic and hydroxylapatite chromatog. Purified recombinant Cyp 2C8 and 2C10 were reconstituted in the presence of NADPH and **NADPH-cytochrome P 450 reductase** and shown to metabolize arachidonic via olefin epoxidn. with both proteins generating, almost exclusively, epoxygenase-derived products (94 and 90% of total products, resp.). Catalytic turnover (1.05 and 0.75 nmol of product/nmol of hemoprotein/min at 30.degree.C for Cyp 2C8 and 2C10, resp.) was inhibited by the addn. of purified cytochrome b5. Metab. by recombinant 2C8 was both regio- and enantioselective for 11(R), 12(S)- and 14(R), 15(S)-epoxyeicosatrienoic acids (82% optical purity, each). Compared to Cyp 2C8, arachidonic acid epoxidn. by Cyp 2C10 was less regio- and stereoselective and generated mixts. of 8(S), 9(R)-, 11(S), 12(R)-, and 14(R), 15(S)-epoxyeicosatrienoic acids (with optical purities of 66, 69, 63%, resp.). Importantly, recombinant Cyp 2C8 and 2C10 epoxidized the arachidonic acid 11, 12-olefin with opposite enantiofacial selectivities. Only for Cyp 2C8 did the chirality of the products match that of the enantiomers present, in vivo, in **human** kidney cortex (A. Karara et al., 1990, FEBS Lett. 268, 227-230). Hence, we propose that Cyp 2C8 is one of the **human** cytochrome P 450 isoforms responsible for the metab. of endogenous arachidonic acid pools.

L7 ANSWER 19 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:596421 HCAPLUS
DOCUMENT NUMBER: 123:27593
TITLE: A NIH/3T3 cell line stably expressing **human cytochrome P450**-3A4 used in combination with a lacZ' shuttle **vector** to study mutagenicity
AUTHOR(S): De Groene, Els M.; Seinen, Willem; Horbach, G. J. M. Jean
CORPORATE SOURCE: Research Institute of Toxicology, Utrecht University, Yalelaan 2, P.O. Box 80.176, TD Utrecht, NL-3508, Neth.
SOURCE: Eur. J. Pharmacol., Environ. Toxicol. Pharmacol. Sect. (**1995**), 293(1), 47-53
CODEN: EPEPEG; ISSN: 0926-6917
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An NIH/3T3 cell line, stably expressing human cytochrome P 450-3A4 (CYP3A4) cDNA has been developed. This cell line was used in combination with a shuttle vector, contg. the bacterial lacZ' gene as reporter gene, to study mutagenicity. Et methanesulfonate and aflatoxin B1 were used as model agents to test this system. The mutation frequency of Et methanesulfonate increased concn. dependently and was the same in CYP3A4-expressing cells as in parental NIH/3T3 cells, demonstrating that CYP3A4 activity has no influence on the mutagenicity of Et methanesulfonate. The mutation frequency of aflatoxin B1 increased concn. dependently only in the CYP3A4-expressing cells and not in parental nor in vector-transfected cells. This increase in mutation frequency could be completely inhibited by ketoconazole, an inhibitor of cytochrome P 450 activity, demonstrating the role of CYP3A4 in the activation of aflatoxin B1. The system described in this paper opens the possibility to study the capacity of single human cytochrome P450s to activate xenobiotics into mutagenic metabolites. Since activation, phase II metab., DNA repair and an endpoint for mutations are all present in one cell, this system will be useful in screening as well as in mechanistic studies.

L7 ANSWER 20 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:550016 HCAPLUS
DOCUMENT NUMBER: 121:150016
TITLE: Isolation and chromosomal localization of the human endothelial nitric oxide synthetase (NOS3) gene
AUTHOR(S): Robinson, Lisa J.; Weremowicz, Stanislawa; Morton, Cynthia C.; Michel, Thomas

CORPORATE SOURCE: Cardiovasc. Div., Brigham and Women's Hosp., Boston, MA, 02115, USA
SOURCE: Genomics (**1994**), 19(2), 350-7
CODEN: GNMCEP; ISSN: 0888-7543
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Nitric oxide (NO) is an important intercellular signaling mol. synthesized in diverse **human** tissues by proteins encoded by a family of NO synthase (NOS) genes. The similarity of sequence and cofactor binding sites has suggested that the NOS genes may also be related to **cytochrome P 450 reductase**, as well as to plant and bacterial oxidoreductases. Endothelial NOS activity is a major determinant of vascular tone and blood pressure, and in several important (and sometimes hereditary) disease states, such as hypertension, diabetes, and atherosclerosis, the endothelial NO signaling system appears to be abnormal. To explore the relation of the endothelial NOS gene to other similar genes, and to delineate the genetic factors involved in regulating endothelial NOS activity, the authors isolated the **human** gene encoding the endothelial NOS. Genomic clones contg. the 5' end of this gene were identified in a **human** genomic library by applying a polymerase chain reaction (PCR)-based approach. Identification of the **human** gene for endothelial NOS (NOS3) was confirmed by **nucleotide** sequence anal. of the first coding exon, which was identical to its cognate cDNA. The NOS3 gene spans at least 20 kb and appears to contain multiple introns. The transcription start site and promoter region of the NOS3 gene were identified by primer extension and RNase protection assays. Sequencing of the putative promoter revealed consensus sequences for the shear stress-response element, as well as cytokine-responsive cis regulatory sequences, both possibly important to the roles played by NOS3 in the normal and the diseased cardiovascular system. The authors also mapped the chromosomal location of the NOS3 gene. First, a chromosomal panel of **human**-rodent somatic cell hybrids was screened using PCR with oligonucleotide primers derived from the NOS3 genomic clone. The specificity of the amplified PCR product was confirmed by **human** and hamster genomic DNA controls, as well as by Southern blot anal., using the NOS3 cDNA as probe. Definitive chromosomal assignment of the NOS3 gene to **human** chromosome 7 was based upon 0% discordancy; fluorescence in situ hybridization sublocalized the NOS3 gene to 7q36. The identification and characterization of the NOS3 gene may lead to further insights into heritable disease states assocd. with this gene product.

L7 ANSWER 21 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:429015 HCAPLUS
DOCUMENT NUMBER: 121:29015
TITLE: High promutagen activating capacity of yeast microsomes containing human cytochrome P-450 1A and human NADPH-cytochrome P-450 reductase
AUTHOR(S): Sengstag, Christian; Eugster, Hans Pietro; Wuergler, Friedrich E.
CORPORATE SOURCE: Inst. Toxicol., Swiss Fed. Inst. Technol., Schwerzenbach, CH-8603, Switz.
SOURCE: Carcinogenesis (**1994**), 15(5), 837-43
CODEN: CRNGDP; ISSN: 0143-3334
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Yeast (Saccharomyces cerevisiae) strains have been constructed that co-express cDNAs coding for the **human** cytochrome P 450 enzymes CYP1A1 or CYP1A2 in combination with **human NADPH-cytochrome P 450 reductase** (oxidoreductase). Microsomal fractions prepd. from the strains were able to efficiently activate various drugs to Salmonella mutagens. These expts. demonstrated that a functional interaction occurred between the resp. **human** enzymes in the yeast microsomes. For every drug tested, the microsomes contg. CYP enzymes and oxidoreductase were 2- to 4-fold better in activation than the corresponding microsomes that contained CYP alone. Interestingly, co-expression of CYP1A2 with

oxidoreductase resulted in a decrease of 7-ethoxyresorufin-O-deethylase activity, a problem which is related to this specific substrate. Using the microsomes, it was demonstrated that aflatoxin B1 was activated to a mutagen not only by CYP1A2 but also by CYP1A1. In contrast, benzo[a]pyrene was exclusively activated by CYP1A2 and to a lesser extend by CYP1A1. Trp-P-2 was activated by CYP1A2 and to a lesser extent by CYP1A1. A strong substrate specificity was obsd. with the two structurally related heterocyclic arylamines MeIQ and MeIQx. MeIQx was activated efficiently by both CYP enzymes, whereas MeIQ was only activated by CYP1A2 and not by CYP1A1. The fact that microsomes from **vector** transformed control strains were unable to activate any of the drugs studied underlies the suitability of these microsomes for metabolic studies.

L7 ANSWER 22 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:97396 HCAPLUS
DOCUMENT NUMBER: 120:97396
TITLE: Expression of **human** placental **cytochrome P450** aromatase (CYP19) cDNA in insect cells using a luciferase based baculovirus **vector**
AUTHOR(S): Lahde, Matti; Raunio, Hannu; Pelkonen, Olavi; Karp, Matti; Oker-Blom, Christian
CORPORATE SOURCE: Orion-Farmos Pharm., Orion Corp., Turku, SF-20101, Finland
SOURCE: Biochem. Biophys. Res. Commun. (**1993**), 197(3), 1511-17
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cDNA encoding human placental cytochrome P 450 aromatase (CYP19) was expressed in Spodoptera frugiperda (Sf9) insect cells using the baculovirus expression vector system. The recombinant protein product was characterized by Northern and Western blot analyses as well as by direct measurement of aromatase activity. The expressed enzyme proved to be both catalytically active in the presence of P 450 reductase and immunol. reactive with polyclonal antibodies raised against human placental aromatase. The activity of aromatase increased 10% after the addn. of 0.1 .mu.g/mL hemin chloride to the culture medium. However, the level of aromatase protein decreased considerably when the concn. of hemin chloride reached 10 .mu.g/mL indicating that hemin chloride has toxic effects on the lepidopteran insect cell line. In conclusion, the baculovirus system is suitable for high level expression of functional human placental CYP19.

L7 ANSWER 23 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:70563 HCAPLUS
DOCUMENT NUMBER: 120:70563
TITLE: Isolation and characterization of a complementary deoxyribonucleic acid insert encoding bovine aromatase cytochrome P450
AUTHOR(S): Hinshelwood, Margaret M.; Corbin, C. Jo; Tsang, Paul C. W.; Simpson, Evan R.
CORPORATE SOURCE: Southwest. Med. Cent., Univ. Texas, Dallas, TX, 75235-9051, USA
SOURCE: Endocrinology (**1993**), 133(5), 1971-7
CODEN: ENDOAO; ISSN: 0013-7227
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Aromatase, an enzyme complex comprised of aromatase cytochrome P 450 (P450arom; the product of the CYP19 gene) and the flavoprotein **NADPH-cytochrome P 450 reductase**, catalyzes the conversion of androgens to estrogens. Three cDNA inserts encoding P450arom were isolated from a bovine placental cDNA library. These inserts were sequenced and found to correspond closely to **human** P450arom sequence from the internal EcoRI restriction site (exon III) through the termination codon (exon X) into the 3'-untranslated region. The rapid amplification of cDNA ends

technique was used to generate the rest of the cDNA 5' of the internal EcoRI site, using mRNA obtained from bovine granulosa cells as a template. This insert was sequenced, and when aligned with the other inserts, an open reading frame was found which was predicted to encode a protein of 503 amino acid residues. The deduced polypeptide shares 84% identity with **human** P450arom and 79%, 76%, 71%, and 57% identity with mouse, rat, chicken, and trout P450arom, resp. A full-length open reading frame was generated using the polymerase chain reaction and mRNA obtained from bovine granulosa cells as template. After this insert was ligated into the pCMV5 expression **vector**, it was transfected into COS-1 monkey kidney tumor cells. The authors were able to demonstrate aromatase activity by assaying the incorporation of tritium into [3H]water from [1.beta.-3H]androsterendione. Northern anal. revealed a single transcript of .apprx.6 kilobases in poly(A)+RNA obtained from bovine placental tissue and granulosa cells. This indicated for the first time a correspondence between the pattern of estrogen biosynthesis throughout the bovine ovarian cycle and the levels of transcripts encoding P450arom. In addn., weak hybridization was noted to transcripts of the expected size, namely 3.4 and 2.9 kilobases, in poly(A)+ RNA obtained from **human** placental tissue. The large size of the bovine transcript is due to a long 3'-untranslated region, because, based on the rapid amplification of cDNA ends technique, there appeared to be approx. 150 basepairs 5' of the start site of translation, and the authors were never able to find a polyadenylation site, even in one clone that went well past the corresponding polyadenylation site in **human** P450arom.

L7 ANSWER 24 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:24675 HCAPLUS
DOCUMENT NUMBER: 120:24675
TITLE: Cloning and sequencing of human liver cytochrome P-450 cDNA clones
AUTHOR(S): Kolyada, A. Yu.
CORPORATE SOURCE: All-Union Cancer Res. Cent., Moscow, 115478, Russia
SOURCE: Cytochrome P-450: Biochem. Biophys., Proc. Int. Conf. Biochem. Biophys. Cytochrome P-450, 7th (**1992*****), **Meeting Date 1991, 455-6. Editor(s): Archakov, A. I.; Bachmanova, G. I. INCO-TNC: Moscow, Russia.**
CODEN: 59FUA9
DOCUMENT TYPE: **Conference**
LANGUAGE: **English**
AB **18 Clones were detected in cDNA library of adult human liver in .lambda.gt11 by immunoscreening. One of these clones (P450HPH) was sequenced. The sequence is essentially identical to the sequence HpI-I and M-12 described for the P 450 IIC8 type gene by S.T. Okino et al. (1987) and C. Ged et al. (1988), resp. The sole difference in the translatable part consists of a A.fwdarw.G substitution at position 528, resulting in a Lys.fwdarw.Arg mutation. There are minor differences in the untranslatable 3'-region (one base substitution at position 817 and one base deletion at position 950). Evidently, P-450HPH sequence is one of the allelic variants of the IIC8 gene.**

L7 ANSWER 25 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: **1993:511899 HCAPLUS**
DOCUMENT NUMBER: **119:111899**
TITLE: **Expression and purification of functional human 17.alpha.-hydroxylase/17,20-lyase (P450c17) in Escherichia coli. Use of this system for study of a novel form of combined 17.alpha.-hydroxylase/17,20-lyase deficiency**
AUTHOR(S): **Imai, Tsuneo; Globerman, Hadas; Gertner, Joseph M.; Kagawa, Norio; Waterman, Michael R.**
CORPORATE SOURCE: **Southwest. Med. Cent., Univ. Texas, Dallas, TX, 75235, USA**
SOURCE: **J. Biol. Chem. (***1993), 268(26), 19681-9**
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymically active **human** 17.alpha.-hydroxylase cytochrome P 450 (P450c17) has been expressed in and purified from Escherichia coli. The cDNA contg. modifications within the amino-terminal eight codons which are favorable for expression in E. coli, as well as codons for 4 histidine residues at the carboxyl terminus, was placed in the pCWori+ expression **vector**. The modified **human** P450c17 was detected spectrophotometrically (400 nmol of P450c17/L culture) and was found to be integrated into E. coli membranes. This previously inaccessible **human** P 450 was purified to electrophoretic homogeneity (10.7 nmol of P 450/mg) from solubilized bacterial membranes using two sequential chromatog. steps, nickel nitrilotriacetate followed by hydroxylapatite. The expected enzymic activities of **human** P450c17 were reconstituted by addn. of purified rat liver **NADPH-cytochrome P 450 reductase**, giving turnover nos. of 8.0 nmol/min/nmol P 450 for pregenolone, 6.5 nmol/min/nmol P 450 for progesterone, 0.06 nmol/min/nmol P 450 for 17.alpha.-hydroxypregnenolone, and no detectable activity for 17.alpha.-hydroxyprogesterone. This system was utilized to study the mol. basis of a novel form of combined 17.alpha.-hydroxylase, 17,20-lyase deficiency resulting from compd. heterozygous mutations, a missense point mutation Tyr64(TAT) .fwdarw. Ser(TCT), and an Ile112 duplication (ATCATC). Upon expression of these mutant proteins in E. coli, the Tyr64 mutant has 15% of the wild type 17.alpha.-hydroxylase activity, whereas the Ile112 duplication shows no activity, results consistent with the obsd. clin. phenotype.

L7 ANSWER 26 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:403860 HCAPLUS
DOCUMENT NUMBER: 119:3860
TITLE: Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase
AUTHOR(S): Janssens, Stefan P.; Shimouchi, Akito; Quertermous, Thomas; Bloch, Donald B.; Bloch, Kenneth D.
CORPORATE SOURCE: Dep. Med., Harvard Med. Sch., Boston, MA, 02114, USA
SOURCE: J. Biol. Chem. (**1992**), 267(21), 14519-22
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Nitric oxide, which accounts for the biol. activity of endothelium-derived relaxing factor (EDRF), is synthesized in endothelial cells from L-arginine by nitric oxide synthase (NOS). Here the cloning and functional expression of a cDNA encoding **human** endothelial NOS is reported. Oligonucleotides corresponding to amino acid sequences shared by **cytochrome P 450 reductase** and the recently identified brain NOS were synthesized to amplify a partial cDNA encoding a bovine endothelial cell NOS-related protein. This partial cDNA was used to isolate a cDNA encoding a **human** vascular endothelial NOS. The translated **human** protein is 1294 amino acids long and shares 52% of its amino acid sequence with brain NOS. Using RNA blot hybridization, abundant endothelial NOS mRNA was detected in unstimulated **human** umbilical vein endothelial cells. To det. the functional activity of the endothelial protein the cDNA was ligated into an expression **vector** and transfected into NIH3T3 cells. Cells expressing this cDNA contained abundant NADPH diaphorase activity, a histochem. marker for NOS. In co-culture assays, nitric oxide prodn. by transfected cells increased guanylate cyclase activity in reporter rat fetal lung fibroblasts. In addn., NOS-catalyzed conversion of arginine to citrulline in transfected cells was significantly increased by A23187, a calcium ionophore. Isolation of a cDNA encoding a calcium-regulated, constitutively expressed **human** endothelial NOS, capable of producing EDRF in blood vessels, will accelerate the characterization of the role of this enzyme in normal and abnormal endothelial regulation of vascular tone.

L7 ANSWER 27 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:401846 HCAPLUS

DOCUMENT NUMBER: 119:1846
TITLE: Detection of single base differences using biotinylated nucleotides with very long linker arms
AUTHOR(S): Livak, Kenneth J.; Hobbs, Frank W.; Zagursky, Robert J.
CORPORATE SOURCE: Du Pont Merck Pharm. Co., Wilmington, DE, 19880-0328, USA
SOURCE: Nucleic Acids Res. (**1992**), 20(18), 4831-7
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A simple primer extension method for detecting nucleotide differences is based on the substitution of mobility-shifting analogs for natural nucleotides. This technique can detect any single-base difference that might occur including previously unknown mutations or polymorphisms. Two tech. limitations of the original procedure have now been addressed. First, switching to Thermococcus litoralis DNA polymerase has eliminated variability believed to be due to the addn. of an extra, non-templated base to the 3' end of DNA by Taq DNA polymerase. Second, with the analogs used in the original study, the mobility shift induced by a single base change can usually be resolved only in DNA segments 200 nt or smaller. This size limitation has been overcome by synthesizing biotinylated nucleotides with extraordinarily long linker arms (36 atom backbone). Using these new analogs and conventional sequencing gels (0.4 mm thick), mutations in the human .beta.-hexosaminidase .alpha. and CYP2D6 genes have been detected in DNA segments up to 300 nt in length. By using very thin (0.15 mm) gels, single-base polymorphisms in the human APOE gene have been detected in 500-nt segments.

L7 ANSWER 28 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:403223 HCAPLUS
DOCUMENT NUMBER: 117:3223
TITLE: Quantification of cytochrome P450 reductase gene expression in human tissues
AUTHOR(S): Shephard, Elizabeth A.; Palmer, Colin N. A.; Segall, H. J.; Phillips, Ian R.
CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Univ. Coll. London, London, WC1E 6BT, UK
SOURCE: Arch. Biochem. Biophys. (**1992**), 294(1), 168-72
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Several cDNA clones that code for a variant of human cytochrome P 450 reductase were isolated and sequenced. An RNase protection assay was used to quantify the corresponding mRNA in adult and fetal tissues. In the samples analyzed, the cytochrome P 450 reductase gene displays very little interindividual variation in its expression in adult liver and is subject to little developmental or tissue-specific regulation.

L7 ANSWER 29 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:104431 HCAPLUS
DOCUMENT NUMBER: 116:104431
TITLE: High-level expression of functional human cytochrome P450 1A2 in Escherichia coli
AUTHOR(S): Fisher, Charles W.; Caudle, Deborah L.; Martin-Wixtrom, Cheryl; Quattrochi, Linda C.; Tukey, Robert H.; Waterman, Michael R.; Estabrook, Ronald W.
CORPORATE SOURCE: Southwest. Med. Cent., Univ. Texas, Dallas, TX, USA
SOURCE: FASEB J. (**1992**), 6(2), 759-64
CODEN: FAJOEC; ISSN: 0892-6638
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymically active **human** cytochrome P 450 1A2 was expressed in E. coli utilizing the pCWori+ **vector** contg. a modified cDNA. The coding sequence for the NH2-terminal region of the protein was modified by the alignment and substitution of a 27-bp segment from a

modified bovine P 450 17A1 cDNA onto the 5' end of the open reading frame of P 450 1A2 at amino acid 21. The expressed chimeric P 450 was produced at a high level in a functionally intact form, as assayed by the formation in vivo of the 449 nm absorbance band of the CO complex of the reduced hemoprotein. E. coli membrane prepns. contain P 450 1A2, which was active in the 2-hydroxylation of estradiol and the O-deethylation of 7-ethoxycoumarin and 7-ethoxyresorufin, when reconstituted with recombinant rat liver **NADPH-cytochrome P 450 reductase**.

L7 ANSWER 30 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:198952 HCAPLUS
DOCUMENT NUMBER: 114:198952
TITLE: Determination of covalent binding to intact DNA, RNA, and oligonucleotides by intercalating anticancer drugs using high-performance liquid chromatography. Studies with doxorubicin and NADPH cytochrome P-450 reductase
AUTHOR(S): Cummings, Jeffrey; Bartoszek, Agnieszka; Smyth, John F.
CORPORATE SOURCE: Imp. Cancer Res. Fund Med. Oncol. Unit, West. Gen. Hosp., Edinburgh, EH4 2XU, UK
SOURCE: Anal. Biochem. (**1991**), 194(1), 146-55
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB An HPLC method is described which can det. covalent binding to intact **nucleic acids** by intercalating anticancer drugs and at the same time remove noncovalently bound intercalated drugs. The method uses a nonporous 2-.mu.m DEAE anion-exchange resin column capable of sepn. of **nucleic acids** >50,000 bases in size in under 1 h. After priming with 1 mg DNA, the column behaves as a intercalator affinity column strongly retaining the drug while allowing the **nucleic acid** to pass through. The retained rug is released with 0.1 M KOH. Incubations were performed with doxorubicin binds covalently to DNA. When [14C]doxorubicin was mixed with DNA at a concn. where all the drug would bind by intercalation, the column retained 82% of the total radioactivity and only 18% migrated with the **nucleic acid**. When the DNA was mildly denatured by 2 M NaCl at 50.degree. for 45 min before chromatog., 99.8% of total radioactivity was retained and only background counts migrated with the **nucleic acid**, as was the case with single-stranded DNA and RNA without any treatment. Purified **NADPH cytochrome P 450 reductase** was used to activate doxorubicin. DNA inhibited the metab. of the drug by the enzyme, no covalent binding occurred with RNA, low levels occurred with single-stranded DNA (34 pmol/100.mu.g), and the highest levels were recorded with oligonucleotides (243 pmol/100.mu.g). The assay was sufficiently sensitive to measure covalent binding to DNA extd. from MCF-7 **human** breast cancer cells treated with 50 .mu.M [14C]doxorubicin (18.6 pmol/100 .mu.g). Thus, covalent binding to DNA, RNA, and oligonucleotides by intercalators can be measured quickly (20 min) without extensive sample prepn. and **nucleic acid** digestion.

L7 ANSWER 31 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:96211 HCAPLUS
DOCUMENT NUMBER: 114:96211
TITLE: Expression of human liver cytochrome P450 IIIA4 in yeast. A functional model for the hepatic enzyme
AUTHOR(S): Renaud, Jean Paul; Cullin, Christophe; Pompon, Denis; Beaune, Philippe; Mansuy, Daniel
CORPORATE SOURCE: Lab. Chim. Biochim. Pharmacol. Toxicol., Cent. Natl. Rech. Sci., Paris, F-75270, Fr.
SOURCE: Eur. J. Biochem. (**1990**), 194(3), 889-96
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cytochrome P 450 (P 450) NF, a member of the P 450 IIIA subfamily, is the

major contributor the the oxidn. of the calcium-channel blocker nifedipine in **human** liver microsomes. A cDNA clone designated NF25 encoding **human** P 450 NF was isolated from bacteriophage .lambda.gt11 expression library (Beaune, P. H., et al. NF25 cDNA was expressed in Saccharomyces cerevisiae using an expression **vector** constructed from pYeDP1/8-2. Yeast transformed with the plasmid contg. the NF25 sequence (pVNF25) showed a ferrous-CO spectrum typical of cytochrome P 450. Microsomal prepns. contained a protein with an apparent mol. mass identical to that of P 450-5 (a form isolated from **human** liver indistinguishable from P 450 NM) that was not present in microsomes from control yeast (transformed with pYeDP1/8-2 alone), as revealed by immunoblotting with anti-P 450-5 antibodies. On the other hand, antibodies raised in rabbits against **human** liver P 450 IIC8-10 and rat liver P 450 IA1 and P 450 IIE1 did not recognize yeast-expressed P 450 NF25. The P 450 NF25 content in microsomes was about 90 pmol/mg protein. Microsomal, yeast-expressed P 450 NF25 exhibited a high affinity for different substrates including macrolide antibiotics, dihydroergotamine and miconazole as shown by difference visible spectroscopy. Microsomal suspensions contg. P 450 NF25 were also able to catalyze several oxidn. reactions that were expected from the activities of the protein isolated from **human** liver, including nifedipine 1,4-oxidn., quinidine 3-hydroxylation and N-oxygenation , and N-demethylation of the macrolide antibiotics erythromycin and troleandomycin. The yeast endogenous **NADPH-cytochrome P 450 reductase** thus couples efficiently with the heterologous P 450 NF25 though its level is far lower than that of its ortholog in **human** liver. Indeed addn. of rabbit liver **NADPH-cytochrome P 450 reductase** increased the oxidn. rates. Rabbit liver cytochrome b5 also caused a marked enhancement of catalytic activities, as had been noted previously for this particular P 450 enzyme in a reconstituted system involving the protein purified from **human** liver. Furthermore, the level of the yeast endogenous cytochrome P 450 (lanosterol 14-demethylase) has been found to be negligible compared to the heterologously expressed cytochrome P 450 (30 times less). Thus, yeast microsomes contg. P 450 NF25 constitute by themselves a good functional model for studying the binding capacities and catalytic activities of this individual form of **human** hepatic cytochrome P 450.

L7 ANSWER 32 OF 40 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER:	1991:37106 HCAPLUS
DOCUMENT NUMBER:	114:37106
TITLE:	Maximizing the expression of mammalian cytochrome P-450 monooxygenase activities in yeast cells
AUTHOR(S):	Urban, P.; Cullin, C.; Pompon, D.
CORPORATE SOURCE:	Cent. Genet. Mol., CNRS, Gif-sur-Yvette, 91198, Fr.
SOURCE:	Biochimie (**1990**), 72(6-7), 463-72 CODEN: BICMBE; ISSN: 0300-9084
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Cytochrome P-450s constitute a superfamily or mono-oxygenases which require the assocn. with specific redox enzymes bound to the endoplasmic reticulum membrane for their activity. Conditions for the functional expression of these mammalian enzymes in yeast cells and the resp. merits and limitations of currently used P 450 expression systems, are considered. The dependence of the mouse P 450 IA1 specific activity on the cytochrome expression level in yeast microsomes is studied and results demonstrate that the low amts. of endogenous **NADPH-cytochrome P 450 reductase** and cytochrome b5 which are naturally present, are limiting for the heterologous monooxygenase activities. The sequences encoding **human** liver cytochrome b5, the native and a modified form of the yeast **NADPH-cytochrome P 450 reductase** were cloned by making use of polymerase chain reaction techniques, over-expressed in yeast as functional forms, and characterized. New **vectors** allowing a high level of mammalian P

450 expression upon induction were also constructed and tested. A strategy for the construction of a co-expression system allowing maximal activity of mammalian cytochrome P-450s is discussed.

L7 ANSWER 33 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:1546 HCAPLUS
DOCUMENT NUMBER: 114:1546
TITLE: Sequence of a human liver cytochrome P-450 cDNA clone
AUTHOR(S): Kolyada, A. Yu.
CORPORATE SOURCE: Lab. Chem. Carcinog., All-Union Cancer Res. Cent., Moscow, 115478, Syria
SOURCE: Nucleic Acids Res. (**1990**), 18(18), 5550
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A nucleotide sequence (P-450 HPH) is presented which has been cloned from a cDNA library of adult human liver in .lambda.gt11 by an immunoscreening method. Polyclonal antibodies against cytochrome P-450 from phenobarbital-pretreated rats were used for screening. The sequence is essentially identical to the sequences Hp1-1 and M-12 described previously for the P-450 IIC8-type gene. The sole difference in the translatable part consists of a A.fwdarw.G substitution at position 356, resulting in a Lys .fwdarw. Arg mutation. There are minor differences in the untranslated 3'-region (one base substitution at position 645 and one base deletion at position 778). Evidently, the P-450 HPH sequence is one of the allelic variants of IIC8 gene.

L7 ANSWER 34 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:173334 HCAPLUS
DOCUMENT NUMBER: 112:173334
TITLE: Characterization of a cDNA encoding a new member of the glucocorticoid-responsive cytochromes P450 in human liver
AUTHOR(S): Schuetz, John D.; Molowa, David T.; Guzelian, Philip S.
CORPORATE SOURCE: Dep. Med., Med. Coll. Virginia, Richmond, VA, 23298-0267, USA
SOURCE: Arch. Biochem. Biophys. (**1989**), 274(2), 355-64
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adult human liver contains a form of cytochrome P 450, termed HLp, that resembles the glucocorticoid-inducible cytochrome P450p in rat liver in its structure, function, and regulation. HLp catalyzes the oxidn. of such clin. important substrates as cyclosporin, nifedipine, erythromycin, and midazolam. Recent evidence, however, suggests that HLp may represent .gtoreq.2 closely related forms of cytochromes P 450, one of which is termed P450nf. To search for addnl. members of the Class III human subfamily of HLp-related genes, a human liver cDNA library cloned in phage vector .lambda.gt1 was screened with oligonucleotides and with a cDNA fragment related to HLp. A full-length cDNA (1709 nucleotides) encoding a new form of human cytochrome P 450 termed HLp2 was isolated similar to HLp. HLp2 appears to represent a distinct gene, as judged by partial sequence anal. of a cloned human gene and by hybridizations of Southern blots, under conditions of varying stringency, with a 3'-portion of HLp cDNA and with an oligonucleotide specific for HLp2. Northern blot anal. revealed that HLp/p450nf was present in all samples of liver mRNA from adult patients not treated with inducers of HLp, whereas HLp2 mRNA was undetectable in more than two-thirds. Human fetal liver RNA contained mRNA species 2.1 and 1.9 kb which hybridized with an HLp2 oligonucleotide. HLp2 represents a 3rd member of the Class III glucocorticoid-responsive gene family that is expressed in both fetal and adult human liver and may account for polymorphism in metab. of clin. important drugs.

L7 ANSWER 35 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:173291 HCAPLUS

DOCUMENT NUMBER: 112:173291
TITLE: Human NADPH-P450 oxidoreductase: complementary DNA cloning, sequence and vaccinia virus-mediated expression and localization of the CYPOR gene to chromosome 7
AUTHOR(S): Yamano, Shigeru; Aoyama, Toshifumi; McBride, O. Wesley; Hardwick, James P.; Gelboin, Harry V.; Gonzalez, Frank J.
CORPORATE SOURCE: Lab. Mol. Carcinog., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Mol. Pharmacol. (**1989**), 36(1), 83-8
CODEN: MOPMA3; ISSN: 0026-895X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cDNA contg. the full coding sequence of human NADPH-P 450 oxidoreductase (I) was isolated and completely sequenced. The cDNA contained 2398 base pairs, including 9 and 358 base pairs of 5'- and 3'-noncoding sequences, resp. The human I protein deduced from the cDNA has 677 amino acids with a mol. wt. of 76,656. The cDNA nucleotide and deduced amino acid sequences displayed 83 and 92% similarities, resp., with those of rat I. By use of somatic cell hybrids, the I gene was regionally localized to human chromosome 7 (7p15-q35). The levels of I protein and mRNA were analyzed in 13 human liver specimens and <3-fold variation was found among the different livers. I cDNA was inserted into vaccinia virus and expressed in cell culture. The cDNA-expressed enzyme was active in reducing the electron acceptor cytochrome c. In addn., I stimulated the enzymic activity of vaccinia virus-expressed human P3450 when both recombinant viruses were used to coinfect human cells in culture. An approx. equal mole level of I and P3450 was required to achieve maximal activity for both ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase.

L7 ANSWER 36 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:590145 HCAPLUS
DOCUMENT NUMBER: 111:190145
TITLE: Identification of an aldehyde dehydrogenase in the microsomes of human polymorphonuclear leukocytes that metabolizes 20-aldehyde leukotriene B4
AUTHOR(S): Sutyak, John; Austen, K. Frank; Soberman, Roy J.
CORPORATE SOURCE: Dep. Med., Harvard Med. Sch., Boston, MA, 02115, USA
SOURCE: J. Biol. Chem. (**1989**), 264(25), 14818-23
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It was previously reported that cytochrome P 450LTB in the microsomes of **human** polymorphonuclear leukocytes (PMN) catalyzes 3 .omega.-oxidns. of leukotriene B4 (LTB4), leading to the sequential formation of 20-OH-LTB4, 20-CHO-LTB4, and 20-COOH-LTB4. The identification of the novel final intermediate, 20-CHO-LTB4, allowed direct anal. of its metab. by PMN microsomes in the presence of adenine **nucleotide** cofactors. Microsomes in the presence of 100 .mu.M NAD or 100 .mu.M NADP converted 1.0 .mu.M 20-CHO-LTB4 to 20-COOH-LTB4 with a Km of 2.4 .mu.M and a Vmax of 813.9 pmol/min/mg for NAD, as compared to 0.12 .mu.M and 5.0 pmol/min/mg for NADPH as a cofactor. The conversion of 1.0 .mu.M of 20-CHO-LTB4 to 20-COOH-LTB4 in the presence of satg. concns. (1.0 mM) of both NAD and NADP was not greater than the reaction in the presence of 1.0 mM of each cofactor sep., indicating that NAD and NADP are cofactors for the same enzyme. Antibody to **cytochrome P 450 reductase** did not inhibit the conversion of 20-CHO-LTB4 to 20-COOH-LTB4. When 1.0 .mu.M 20-OH-LTB4 was added to microsomes in the presence of NADPH, .apprx.75% of the product formed (63.7 pmol) was 20-CHO-LTB4 and .apprx.25% (21.3 pmol) was 20-COOH-LTB4. In the presence of both NADPH and NAD, only 20-COOH-LTB4 (85.5 pmol) was formed. PMN microsomes also contained a NADH-dependent aldehyde reductase which converted 20-CHO-LTB4 to 20-OH-LTB4, a member of the LTB4 family of mols. with biol. activity. Based upon kinetic, cofactor and inhibition data, microsomal aldehyde dehydrogenase preferentially regulates the final

and irreversible inactivation step in the LTB4 metabolic sequence.

L7 ANSWER 37 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:472304 HCAPLUS
DOCUMENT NUMBER: 111:72304
TITLE: Isolation of a new human fetal liver cytochrome P450 cDNA clone: evidence for expression of a limited number of forms of cytochrome P450 in human fetal livers
AUTHOR(S): Komori, Masayuki; Nishio, Kanako; Fujitani, Tomomichi; Ohi, Hiroaki; Kitada, Mitsukazu; Mima, Satoaki; Itahashi, Koshiro; Kamataki, Tetsuya
CORPORATE SOURCE: Fac. Pharm. Sci., Hokkaido Univ., Sapporo, 060, Japan
SOURCE: Arch. Biochem. Biophys. (**1989**), 272(1), 219-25
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new cDNA clone (.lambda.HFL10) was isolated from a human fetal liver cDNA library using an antiserum to P-450 HFLa, which was previously isolated from human fetal livers. Cytochrome P-450 cDNAs .lambda.hPA6,.lambda.hP2-1, and .lambda.hPD4 which were highly homologous to cDNA clones pHY13, Hp1-1 and phP450j, resp., were isolated from the cDNA library of human adult livers. Using these cDNA clones as probes together with .lambda.HFL10, Northern blot anal. was conducted to det. whether all of these cytochromes were expressed in human fetal livers. The results clearly showed that only P-450 HFL10 mRNA was detected in human fetal livers. This result supports the contention that cytochrome P-450 polymorphism is less in human fetal livers than in adult livers.

L7 ANSWER 38 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:451404 HCAPLUS
DOCUMENT NUMBER: 111:51404
TITLE: Cloning and structure of the human adrenodoxin gene
AUTHOR(S): Chang, Chi Yao; Wu, Du An; Lai, Char Chang; Miller, Walter L.; Chung, Bon Chu
CORPORATE SOURCE: Inst. Mol. Biol., Acad. Sin., Taipei, 11529, Taiwan
SOURCE: DNA (**1988**), 7(9), 609-15
CODEN: DNAADR; ISSN: 0198-0238
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Adrenodoxin is an iron-sulfur protein that serves as an electron transport intermediate for all mitochondrial forms of **cytochrome P450**. To facilitate studying the regulation of adrenodoxin, the **human** adrenodoxin gene was cloned. It spans more than 20 kb, contg. 4 exons and 3 introns. The first exon encodes the 60-amino-acid signal peptide, directing transport of the protein into the inner mitochondrial matrix. The mature peptide of 124 amino acids is encoded by the other 3 exons. The third exon encodes the portion of the protein contg. the iron-sulfur center and a domain which binds other components of the electron transport chain. The transcriptional start sites were detd. by primer extension and S1 nuclease mapping. The 5'-flanking region of this gene contains canonical promoters including a TATA box at **nucleotide** position -30 and 2 GC boxes at **nucleotide** positions -60 and -100. The sequence at **nucleotides** -234 to -252 is also highly homologous to the glucocorticoid-responsive element and the estrogen-responsive element.

L7 ANSWER 39 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:418739 HCAPLUS
DOCUMENT NUMBER: 111:18739
TITLE: Expression of a human P-450IIC gene in yeast cells using galactose-inducible expression system
AUTHOR(S): Yasumori, Toshio; Murayama, Norie; Yamazoe, Yasushi; Abe, Akio; Nogi, Yasuhisa; Fukasawa, Toshio; Kato, Ryuichi
CORPORATE SOURCE: Sch. Med., Keio Univ., Tokyo, 160, Japan

SOURCE: Mol. Pharmacol. (**1989**), 35(4), 443-9
CODEN: MOPMA3; ISSN: 0026-895X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cDNA of a **human** liver cytochrome P 450, corresponding to P 450 **human**-2, was expressed in Saccharomyces cerevisiae cells by the use of a galactose-inducible expression **vector** contg. the GAL7 promoter and terminator. In Western blots using anti-P 450 **human** -2 IgG, a single band, which exhibited mobility identical to that of authentic P 450 **human**-2 purified from **human** liver, was detected in microsomes of the yeast cells. The amt. synthesized in yeast was estd. to be approx. 1% of the total cell protein, and approx. 25% of the cytochrome existed in the holoenzyme state. Microsomes from the P 450 **human**-2-producing yeast showed a catalytic activity towards benzo(a)pyrene, and the activity was significantly enhanced by the addn. of purified **NADPH-cytochrome P 450 reductase**. The yeast microsomes also catalyzed (S)-mephenytoin 4-hydroxylation but not the demethylation. The present results indicate that the yeast cells contg. P 450 **human**-2 cDNA synthesize a functionally active form of the enzyme, the chem. and catalytic properties of which are identical to those of the **human** liver prepn.

L7 ANSWER 40 OF 40 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:167678 HCAPLUS
DOCUMENT NUMBER: 110:167678
TITLE: NADPH-dependent drug redox cycling and lipid peroxidation in microsomes from human term placenta
AUTHOR(S): Byczkowski, Janusz Z.; Kulkarni, Arun p.
CORPORATE SOURCE: Coll. Public Health, Univ. South Florida, Tampa, FL, 33612, USA
SOURCE: Int. J. Biochem. (**1989**), 21(2), 183-90
CODEN: IJBOBV; ISSN: 0020-711X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB NADPH-dependent Fe and drug redox cycling, as well as lipid peroxidn. were investigated in microsomes isolated from **human** term placenta. Paraquat and menadione undergo redox cycling, catalyzed by **NADPH :cytochrome P 450 reductase** in placental microsomes. The drug redox cycling initiates microsomal lipid peroxidn. in the presence of micromolar concns. of Fe and EDTA. O2- was essential for the microsomal lipid peroxidn. in the presence of Fe and EDTA. Drastic peroxidative conditions involving O2- and prolonged incubation in the presence of Fe destroys flavin **nucleotides**, inhibits **NADPH:cytochrome P 450 reductase**, and inhibits the propagation step of lipid peroxidn. Reactive oxo complex formed between Fe and O2- is proposed as an ultimate species for the initiation of lipid peroxidn. in microsomes from **human** term placenta as well as for the destruction of flavin **nucleotides** and inhibition of **NADPH:cytochrome P 450 reductase** as well as for impairment of promotion of lipid peroxidn. under drastic peroxidative conditions.